AD-A209 898

DOCUMENTATION PAGE

Form Approved
OMB No 0704 0188

1a REPORT SECURITY CLASSIFICATION: (U)	1b RESTRICTIVE MARKINGS: N/A
2a SECURITY CLASSIFICATION AUTHORITY: N/A	3 DISTRIBUTION/AVAILABILITY OF REPORT: Distribution Unlimited
2b DECLASSIFICATION/DOWNGRADING SCHEDULE: N/A	
4 PERFORMING ORGANIZATION REPORT NUMBER(S): N/A	5 MONITORING ORGANIZATION REPORT NUMBER(S): N/A
6a NAME OF PERFORMING ORGANIZATION: The Presidents and Fellows of Harvard College; 6b OFFICE SYMBOL (If applicable): NA	7a NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c ADDRESS (City, State, and ZIP Code): Biological Laboratories, 16 Divinity Ave., Cambridge, MA 02138	7b ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000
8a NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research; 8b OFFICE SYMBOL (If applicable): ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-88-K-0130
8c ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 2217-5000	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO: ; PROJECT NO: 1141MB; TASK NO: 4412033; WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification): Molecular Biology and Genetic Regulation in Marine Dinoflagelletes

12 PERSONAL AUTHOR(S): J. W. Hastings (P.I., L. Fritz, T. Lewis

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 880601 TO 890531	890626	3

16 SUPPLEMENTARY NOTATION: N/A

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Dinoflagellates, DNA organization, gene regulation, transcriptional and translational control, nuclear proteins, gene cloning, DNA sequencing.

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Our studies are concerned with the structure, organization and expression of the genome of marine dinoflagellates; they commonly occur as free living photosynthetic marine unicells, but also include endosymbiotic, parasitic and heterotrophic taxa. Some are bioluminescent; some produce potent neurotoxins; and some cause red tides. The dinoflagellate nucleus is unusual in that the chromosomes remain condensed throughout interphase and lack nucleosomes and histone like proteins.

We have undertaken the cloning and structure determination of five selected dinoflagellate genes, those in the bioluminescent system coding for luciferase and luciferin binding protein, and those for nitrate reductase, alpha and beta tubulin. cDNA libraries have been constructed; a cDNA for binding protein has been partially sequenced. Presumptive cDNAs for other genes have also been isolated and are being sequenced. Utilizing appropriate sequences for the synthesis of primers, genomic sequences are being prepared with the polymerase chain reaction technique. Keywords:

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)
22a NAME OF RESPONSIBLE INDIVIDUAL: Michael T. Marron	22b TELEPHONE (Include Area Code): 202/696-4760; 22c OFFICE SYMBOL: ONR

DD Form 1473, JUN 86 — Previous editions are obsolete — SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

89 6 30 024

Annual Report on Contract# N00014-88-K-0130

Date: 26 June 1989

Title: Molecular Biology and Genetic Regulation in Marine Dinoflagellates

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26 June 1989

Research Objectives:

Our long range goals are to understand the organization, regulation and expression of the marine dinoflagellate genome. Although dinoflagellates are abundant in the marine ecosystem, relatively little is known about their genetic makeup. A distinguishing feature of their DNA is that it has very little associated histone type protein, and it may be asked whether the arrangement and/or regulation of genes differ. To that end we are isolating and characterizing several selected dinoflagellate nuclear genes. Sequence information on these genes will enable us to determine intron/exon organization, gene copy numbers and regulatory sequences.

Accomplishments (Year 1)

(1) Sequencing: Luciferin binding protein (LBP is a protein of moderate abundance (~1% of soluble protein) in the marine dinoflagellate Gonyaulax *polyedra*. It functions in the bioluminescent reaction to bind and then release the substrate luciferin; the luciferase reaction then generates the flashes of light emitted by the organism.

At the time this project was initiated a partial (1100 bp) cDNA for the LBP had just been isolated. Based on the size of the protein (72 kDa), the coding region should be about 1.7 kb, and the corresponding mRNA is 2.4 kb, as determined by Northern blotting. We first undertook to determine the nucleotide sequence for this cDNA, and have now completed portions from both ends. From the 3' end a 320bp run has been sequenced; it includes a poly A^+ tail region, and stop codons (for all three reading frames), and an untranslated region. The actual reading frame for LBP has not been determined. Starting from the 5' end, a 290 bp run has been sequenced. For neither of these runs do we find regions homologous with sequences in other genes, based on searches of gene bank data.

(2) Preparation and screening of a new cDNA library: Between the time of the submission of proposal and that start up of funding, a new and better method for obtaining nuclear gene sequences was developed. In particular, the polymerase chain reaction (PRC) methods can now be used, based on sequences determined from cDNAs. We thus altered our plans so as to be able to make use of this technique, and we decided to prepare a new cDNA library, hopefuliy containing longer sequences. This was successful. mRNA was extracted from *G. polyedra* and the poly A^+ fraction isolated on an oligo dT column. cDNA was synthesized with reverse transcriptase using a modification of the Gubler and Hoffman (1983) procedure. Linker-adapters were added and the cDNA was size-selected (1-3kb, >3kb) and ligated into a modified lambda phage vector (λ ZAP II,

Invitrogen). The library has been screened for the LBP cDNA using an oligolabled LBP probe (methods of Feinberg and Vogelstein, 1983;1984) obtained from the earlier cDNA. A new longer LPBc DNA, approximately 1.7 kb, has been isolated.

(3) Cloning of tubulin and nitrate reductase genes: In parallel with the studies of genes concerned with the bioluminescent reaction we undertook studies of other genes, assuming that their regulation might not be the same. We selected tubulin and nitrate reductase. Although cloning has not been completed, considerable progress has been made with both, as described below.

Nitrate reductase gene: The major source of nitrogen in the marine ecosystem is in the form of nitrate . Marine algae must reduce this to ammonia or amines before being used by a reduction pathway beginning with the NADH-depdendent enzyme nitrate reductase (NR). NR was isolated from *Gonyaulax* cells and purified on SDS-PAGE. Polyclonal antibody was rased in rabbits and has been used to screen for the NR cDNA with the new library in an expression vector (Lambda Zap II)

Tublin (alpha and beta): An alpha tubulin cDNA probe from *Chlamydomonas reinhardtii* has been used to screen the new *Gonyaulax* cDNA libraryI. Positive signals have been obtained.

Similar screening with a *Chlamydomonas* probe for beta tubulin was not successful; virtually all the colonies gave a signal, possibly because of hybridization with vector sequences. We thus decided to use the polymerase polymerase chain reaction (PCR) technique as an alternative route (see, e.g., Erlich, H.A., Gelfand, D.H., and Saiki, R.K. Nature 331:461, 1988). Using sequences found in the Genbank sequence database, we identified highly conserved regions of the coding sequence among many organisms. The consensus sequences from two such regions, one at the interior of the message, and one close to the 3' end, were used as a basis for the synthesis of two oligodeoxynucleotides, which have been used as primers in PCR runs. The DNA fragments produced in these reactions are of the expected size.

WORK PLAN (Year 2): We will complete to screening the cDNA library and isolate full length cDNAs for LBP, luciferase, tublin and NR. Based on selected nucleotide sequences, we will synthesize by PCR the full length genes coding for these proteins, including the untranslated regulatory regions. Once obtained, we will sequence these genes, compare these sequences with known gene sequences from other organisms and determine intron/exon structure. The regulatory regions, structures and conformations, will receive particular attention.

Publications: Manuscripts describing the results of this work are planned but not yet prepared.